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THE MUTAGENIC POTENTIAL OF: CHLOROQUINE PHOSPHATE USP.(U)
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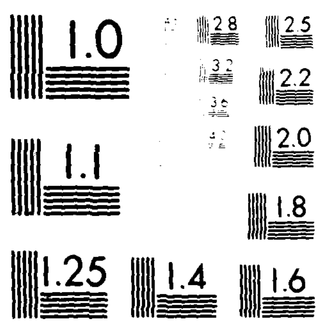

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INSTITUTE REPORT NO. 117

THE MUTAGENIC POTENTIAL OF: chloroquine phosphate USP

LEONARD J. SAUERS, BA, SP5
and
JOHN T. FRUIN, DVM, PhD, COL VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

MARCH 1982 **Toxicology Series 27**

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

THE MUTAGENIC POTENTIAL OF 2,4-DIAMINO-6-(2'-NAPTHYL-SYLFONYL) QUINAZOLINE

L. J. Sauers and J. T. Fruin Toxicology Series: 28

In conducting the research described in this report, the investigation adhered to the "Guide for the Care and Use of Laboratory Animals," as promulgated by the Committee on Revision of the Guide for Laboratory Animal Facilities and Care, Institute of Laboratory Animal Resources, National Research Council.

John D. Marshall Jr. 17 Feb 1982
(Signature and date)

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of chloroquine phosphate USP (WR1522*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 1 mg/plate to 3.2×10^{-4} mg/plate. It was determined that the tested substance did not have mutagenic potential.

*Code Number of Compound

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ABSTRACT

The mutagenic potential of Chloroquine Phosphate USP (WR1544*) was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were exposed to doses ranging from 1 mg/plate to 3.2×10^{-4} mg/plate. It was determined that the test substance did not have mutagenic potential.

* Code number for compound.

PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Fort Detrick, Frederick, MD 21201
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: US Army Medical Research and Development Command
Division of Experimental Therapeutics
Walter Reed Army Institute of Research, Bldg 40
Washington, D.C. 20012

PROJECT/WORK UNIT/APC: 3S162734A875BD, Medical Systems in Chemical Defense, WU 302 Good Laboratory Training, APC TL07

GLP STUDY NUMBER: 81031

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: SP5 Leonard J. Sauers, BA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocol, and retired SOPs will be retained in the LAIR Archive. Test compounds were provided by the sponsor. Chemical, analytical, purity, etc. data are available in Appendix A.

TEST SUBSTANCE: Chloroquine Phosphate USP
Vehicle - DMSO

INCLUSIVE STUDY DATES: October - November 1981

OBJECTIVE: To determine the mutagenic potential of the above compound using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. The plate incorporation method was followed. The test substance was dissolved in DMSO and this diluent was checked for sterility.

ACKNOWLEDGMENTS

The authors wish to thank John Dacey, SP4 Lawrence Mullen, BS and SP4 Thomas Kellner, BA for their assistance in performing the research.

Signatures of Principal Scientists involved
in the Study

We, the undersigned, believe the study described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply to the best of our ability with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

Leonard J. Sauers 19 Feb 82	John T. Fruin 22 Feb 82
LEONARD J. SAUERS, BA/DATE	JOHN T. FRUIN, DVM, PhD/DATE
SP5	COL, VC
Principal Investigator	Study Director

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA 23 November 1981

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 81031 the following inspections were made:

5 October 1981
7 October 1981
9 October 1981
14 October 1981
22 October 1981

The report and raw data for this study were audited on 23 November 1981.

Inspection findings were reported to the Study Director on 15 October 1981. These inspections will also be included in the December 1981 report to management.

John C Johnson
JOHN C. JOHNSON
CPT, MS
Quality Assurance Officer

TABLE OF CONTENTS

THE MUTAGENIC POTENTIAL OF: chloroquine phosphate USP
Toxicology Series 27--Sauers and Fruin

This test substance is a candidate anti-malarial drug tested by the Ames Assay at the request of the Walter Reed Army Institute of Research. The plate incorporation method was followed and the chemical was dissolved in DMSO. The DMSO was checked for sterility.

Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to in vivo tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsome enzyme system to increase sensitivity through enzymatically altering the test substance into a potentially metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of *Salmonella typhimurium*, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon back to the wild type and thus reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon`s mutation and is able to induce point mutations and genetic damage (2).

In order to increase the sensitivity of the test system, two other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysacchride layer (LP) is mutated and allows larger molecules to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. Since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic

process, a mammalian microsome system is incorporated. These microsomal enzymes are obtained from livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used method to monitor the integrity of the organisms, and data pertaining to current and historical control and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occcur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases; exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a larger number of revertants are obtained, then the bacteria is adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs spontaneously with the running of each assay. The value of the spontaneous reversion rate is obtained using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at -80 C in our laboratory. Before any substance was tested, quality controls were run on the bacterial strains to establish the validity of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred.

We compare the spontaneous reversion values with our own historical values and these cited by Ames, et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538 and TA 98) (2).

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 is used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontanecus reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mgplate is used when no toxicity is observed. The densities were recorded as normal slight, and no growth.

Test Format

After we have validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1 ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance are added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains are used 16 hours (maximum) after initial inculation into nutrient broth. The dose of the test substance spanned a 1000-fold, decreasing from the minimum toxic level by a dilution factor of 5. All the substances were tested with and

without S-9 microsome fraction. The S-9 mixture which was previously titered at an optimal strength was added to the molten top agar. After all the ingredients were added, the top agar was vortexed, then overlayered on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated, upside down in the dark at 37 C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistially. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen: Assay of over 300 Chemicals," have concurred on the test`s ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by the Ames method. He assumes that a compound which causes twice the reversion rate experimentally as spontaneously, is mutagenic.

Chemical Analysis

Our information on the chemcial analysis was obtained from Chun and Lam (Appendix A). WR1544 · $2H_3PO_4$ (Chloroquine Phosphate USP) Lot AG, Bottle Number AJ20618.

RESULTS AND DISCUSSION

Throughout this report Chloroquine phosphate will be referred to by its respective code number WR1544.

On 1 October 1981, the Toxicity Level Determination was performed on the test substance. For this experiment, all sterility, strain verification, and negative controls were normal (Table 1). No toxicity was observed at the highest dose of 1 mg/plate (Table 2).

Two Ames Assays were run to determine conclusively the mutagenic activity of WR1544. The initial assay performed on 6 October 1981 showed normal results to all strain verification and sterility controls (Table 3). An unexpected response was observed for the spontaneous reversion rates taken at the end of the assay. An unexpected response by TA 100 to positive control chemical dimethyl benzanthracene (DMBA) was also observed (Table 4). In response to WR1544, there were several incidences of no growth and abnormally low reversion counts associated with TA 98, and TA 1538 (Table 5). Due to these irregular results, a second assay was run on 22 October 1981.

In the second experiment, all sterility and strain verification controls were normal (Table 6). The spontaneous reversion rate and all positive controls were normal except the response of TA 1538 to DMBA (Table 7). A few scattered incidences of a more than doubling of the spontaneous reversion rate were seen in response to the test chemical. These were seen for activated TA 1537 at the 0.2 and 0.04 mg/plate dose and nonactivated at the 0.2 mg/plate dose. No dose response was seen (Table 8).

CONCLUSION

The Ames Test is able to detect frameshift and basepair mutagenic potential. Our results show no evidence of such potential. Therefore, on the basis of the Ames test, Chloroquine phosphate, both in the presence and absence of metabolic activation, is not mutagenic at the levels tested.

RECOMMENDATION

None

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4. VOGEL, H. J. and D. M. BONNER. Acetylornithinase of E. coli: Partial purification and same properties, J Biol Chem, 218: 97-106, 1956

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Report on Chemical Analysis

APPENDIX A

APPENDIX A

Assay of 7-Chloro-4-{[4-(diethylamino)-1-methylbutyl]-amino}quinoline diphosphate, Chloroquine Phosphate, WR-1544, BG58689

Report No. 357
21 June 1979

Contract No. DADA 17-73-C-3171
SRI International Project No. 2772

Report Accepted
By
Date 1/15/80

RECEIVED JAN 15 1980

For
Headquarters, U.S. Army Medical
Research and Development Command
Office of the Surgeon General
Washington, D. C. 20314

APPENDIX A, continued

Preface

This report was prepared at SRI International, 333 Ravenswood Avenue, Menlo Park, California, 94025, under U.S. Department of the Army, Contract No. DADA 17-73-C-3171, SRI International Project No. 2772, "Analysis of Organic Chemical Products, Investigational New Drugs and Composition Thereof." This project was supported by the Division of Experimental Therapeutics, Walter Reed Army Medical Center, Walter Reed Army Institute of Research (WRAIR), U.S. Army Medical Research and Development Command. Melvin Heiffer, Ph.D., of the Pharmacology Department is the project monitor.

This work was conducted in the Life Sciences Division by Mrs. Jane Chiu, chemical technician and Mr. Allen Benitez, chemist, between 1-14 June 1979.

Jane Chiu

Jane Chiu, Chemical Technician

Allen Benitez /PL

Allen Benitez, Chemist

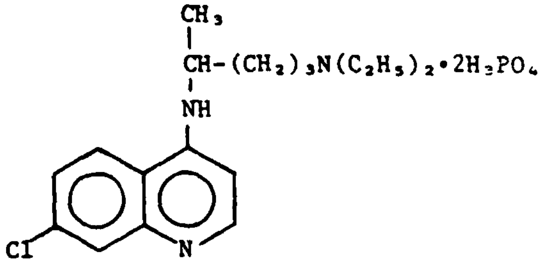
Peter Lim

Peter Lim, Principal Investigator

APPENDIX A, continued

Assay of 7-Chloro-4-{[4-(diethylamino)-1-methylbutyl]-amino}quinoline diphosphate, Chloroquine Phosphate, WR-1544, BG58689

Report No. 357



$C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$ 515.87

Objective

The objective of this investigation was to confirm the identity and to establish the purity of the WR-1544, BG58689 sample.

Summary

On the basis of comparative spectral and chromatographic evidence, the principal component in the sample has been verified as chloroquine phosphate.

Based on the USP assay, the sample is 93.9% pure; however, the carbon content of the sample is only 93.3% of the calculated. The sample also contains 3.2-3.8% water. Taking into consideration the water (3.5%) and calculating sample purity on a dried basis, the respective percentages become 97.3 and 96.7. The U.S. Pharmacopeia XIX requires a dried purity percentage of 98-102.

In addition, the sample contains a water-insoluble, and apparently non-carbonaceous substance (∿ 2.9 to 3.5% by difference). Thin-layer chromatographic data show four very minor to trace impurities; one is uv-absorbing and three are fluorescent. The USP chloroquine phosphate reference standard also shows four very minor to trace impurities: three of these appeared chromatographically alike to those found in the subject sample.

Introduction

The sample was received on 21 May 1979 and is covered by a letter from Dr. Melvin H. Heiffer dated 9 May 1979. Experimentals are recorded in Notebook No. 2979.

Experimentals

A. Tests according to the U.S. Pharmacopeia XIX, page 82.

Description. The sample is odorless, white, and granular.

pH (1% solution). 4.9; USP Chloroquine phosphate, 4.8. USP XIX requires pH $\sim$ 4.5.

Melting Range (Mel-temp apparatus, 1°C/min, capillary applied at 183°C).

189-209 (dec., corrected)

187-191 (dec., corrected, USP Chloroquine Phosphate Reference Standard)[1]

Solubility. Except for a small portion, the sample is soluble in water. The water-insoluble portion did not dissolve by increasing the water volume or by heating.

Identification. The ultraviolet absorption spectrum (Fig. 1) of a 1 in 25,000 solution of dilute hydrochloric acid (1 in 1000) exhibits maxima and minima at the same wavelengths as that of a similar solution of USP Chloroquine Phosphate Reference Standard, comcomitantly measured. The A_{343}/A_{329} ratio is 1.09, which is exactly the same for the USP Chloroquine Phosphate Reference Standard. USP XIX gives a ratio range of 1.00-1.15.

Loss on Drying (100° C, 1 mm Hg, 5 hours).

3.3% from a 306.3-mg sample

Assay. The sample (100 mg) was dissolved in 5 ml of water with the aid of gentle sonication, and the "solution" was centrifuged to allow the insolubles to settle. The supernatant was diluted (1 to 500) with dilute hydrochloric acid (1 in 1000). The diluted solution was assayed by ultraviolet measurements at 343 and 329 nm.

The results obtained were equated to a reference value, analogously derived from USP Chloroquine Phosphate Reference Standard. The reference

---

[1] Merck 8th ed. reports chloroquine phosphate to be dimorphic; one form melts 193-195°, the other melts 215-218°.

standard purity was corrected to 99.9% because of an impurity (∿ 0.1%[a]) found by tlc. There appear to be three more very minor impurities present, but their quantities could not be estimated because of their fluorescent characteristics. The sample purity reported for each wavelength is the average of four separate determinations.

λ		Sample Purity
343		93.9, s = 0.9
329		93.8, s = 0.9
	Average	93.9, s = 0.9

B. Analyses Not Required by USP XIX

An infrared absorption spectrum (Fig. 2), recorded as a Nujol mull, compares very well with the spectrum of USP Chloroquine Phosphate Reference Standard (Fig. 2A).

Proton-magnetic-resonance spectrum (Fig. 3), which was determined as a D_2O solution, is qualitatively and quantitatively consistent with the structure of chloroquine phosphate.

Elemental Analysis

	%C	%H	%Cl	%N	%P
Calcd. for $C_{18}H_{26}ClN_3 \cdot 2H_3PO_4$	41.91	6.25	6.87	8.15	12.01
Found	39.12	6.06	6.30	7.63	11.01
Percentage of found/calcd.	93.3	97.0	91.7	93.6	91.7

Karl Fischer Water Determination

$$\left.\begin{array}{l}\text{3.2\% from a 71.17-mg sample}\\ \text{3.8\% from a 21.71-mg sample}\end{array}\right\}\ \text{Average 3.5\%}$$

Thin-Layer Chromatography

Adsorbent. Commercially prepared $SiO_2 \cdot GF$, Analtech, Inc. (0.025 x 20 x 20 cm)

Quantities spotted. 100, 200, and 500 µg (100 µg/µl H_2O, supernatant from centrifuged solution)

Reference samples. USP Chloroquine Phosphate Reference Standard
Ammonium phosphate (Baker, lot 8648)

[a]This estimation assumes comparable tlc responses from chloroquine and this impurity.

Detection. (a) uv (254 and 365 nm)

(b) Iodine vapor

(c) Spray in sequence - 1% ammonium molybdate and 1% stannous chloride in 10% HCl

Solvent system (solvent front travelled 13 cm)

MeOH/conc. NH_4OH, 58% (25/1, v/v)

Results. The sample solutions were prepared, quickly spotted under subdued light, and the plates were developed in darkened tanks in order to eliminate or reduce photodecomposition.[3]

The subject sample was resolved into the following spots: R_fs 0.00 (ammonium phosphate; an impurity is indicated by 365 nm light and by iodine vapor), 0.07 (very minor, detected by 365 nm light on a damp plate; spot disappears when plate dries), 0.27 (∿ 0.2%[4]), 0.47 (major, WR-1544), and 0.57 (very minor, detected by 365 nm light on a damp plate; spot disappears when plate dries).

The chromatogram of the USP Chloroquine Phosphate Reference Standard displayed the same spots at R_f 0.00 (less intense under 365 nm light than for subject sample; does not respond to iodine vapor), 0.27 (∿ 0.1%[4]), 0.47 (major, WR-1544), and 0.57. A spot at R_f 0.17 (detected under 365 nm light on a damp plate; spot disappears when plate dries) was novel to the reference sample.

Isolation of Water-Insoluble Material from Sample

The water-insoluble material that remained after the dissolution of the chloroquine phosphate was collected, washed with water and dried. Its infrared spectrum (Fig. 4) indicates an inorganic material.

Conclusion

The subject sample is chloroquine phosphate of 93 ± 1% purity.

It also contains 3.5 ± 0.3% water, four very minor to trace unknown impurities indicated by thin-layer chromatographic data, and a water-insoluble, apparently noncarbonaceous material estimated at ∿ 3.5% (by difference).

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[3] If these precautions were not exercised, artifacts, appearing as additional minor components on the chromatogram, would be found.

[4] Estimation based on visual comparisons against known quantities of USP Chloroquine Phosphate Reference Standard under 254 nm light.

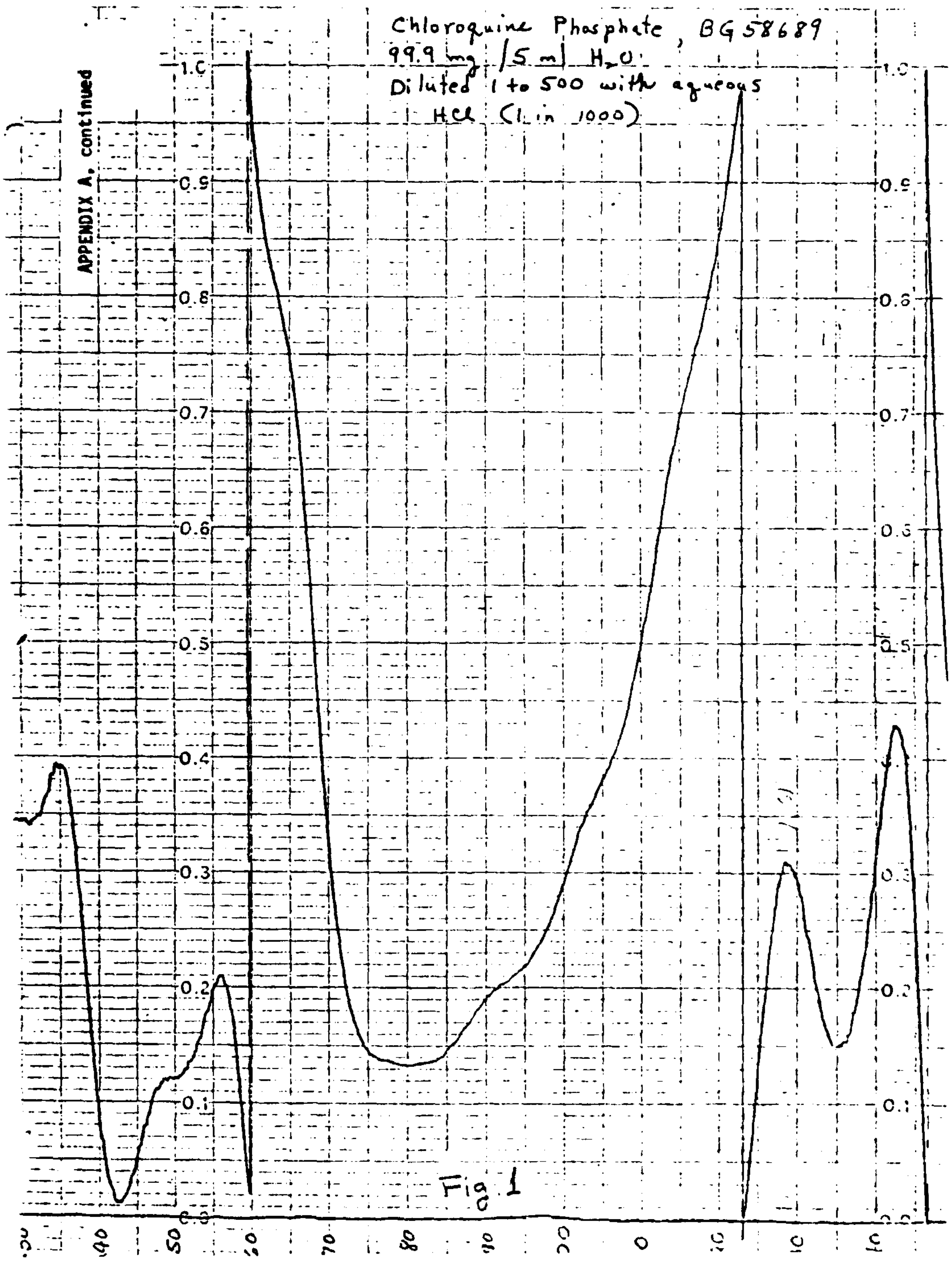
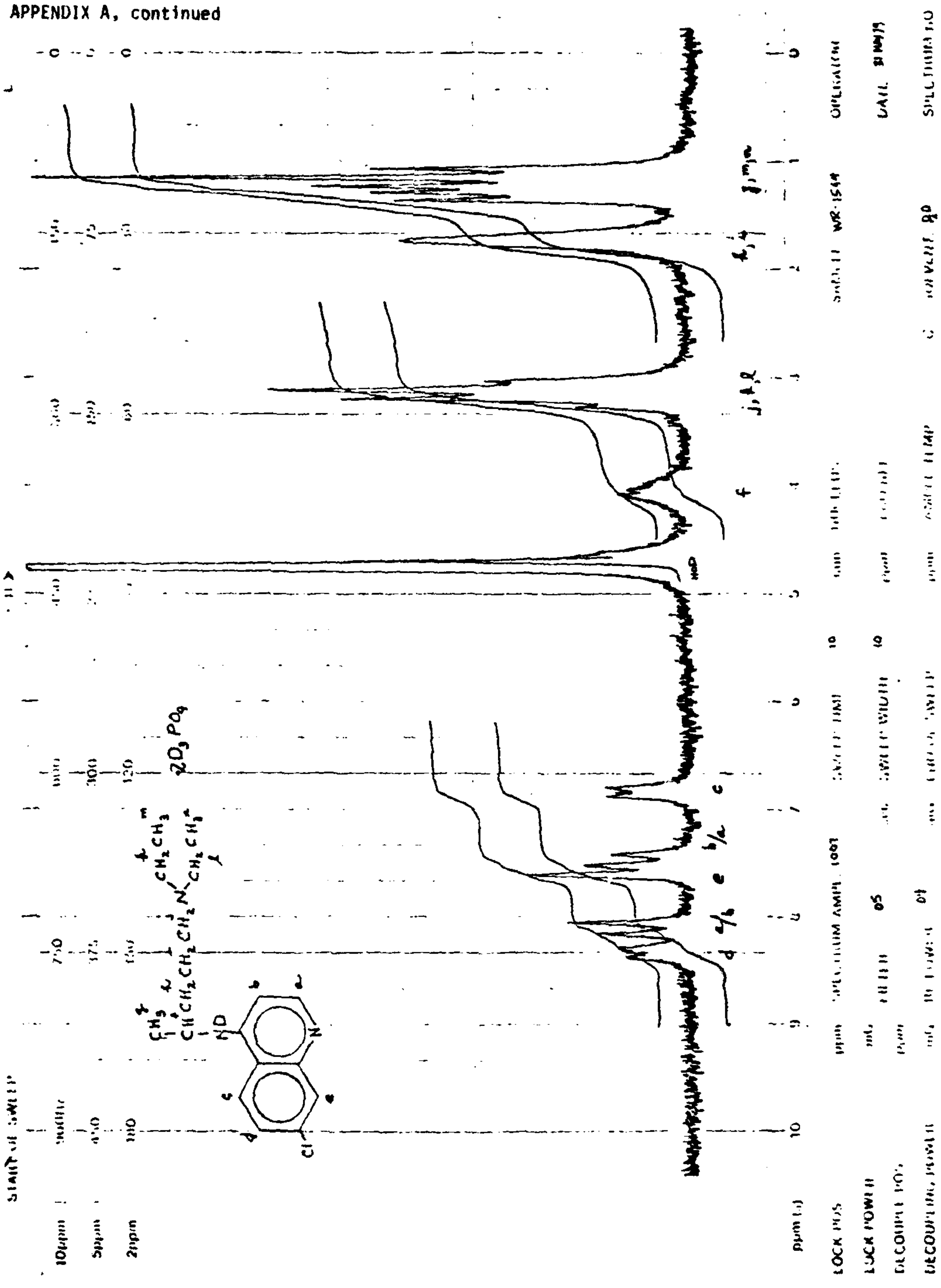

Chloroquine Phosphate, BG 58689
99.9 mg /5 ml H_2O
Diluted 1 to 500 with aqueous
HCl (1 in 1000)
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
40
50
60
70
80

Fig 1

APPENDIX A, continued



APPENDIX A, continued

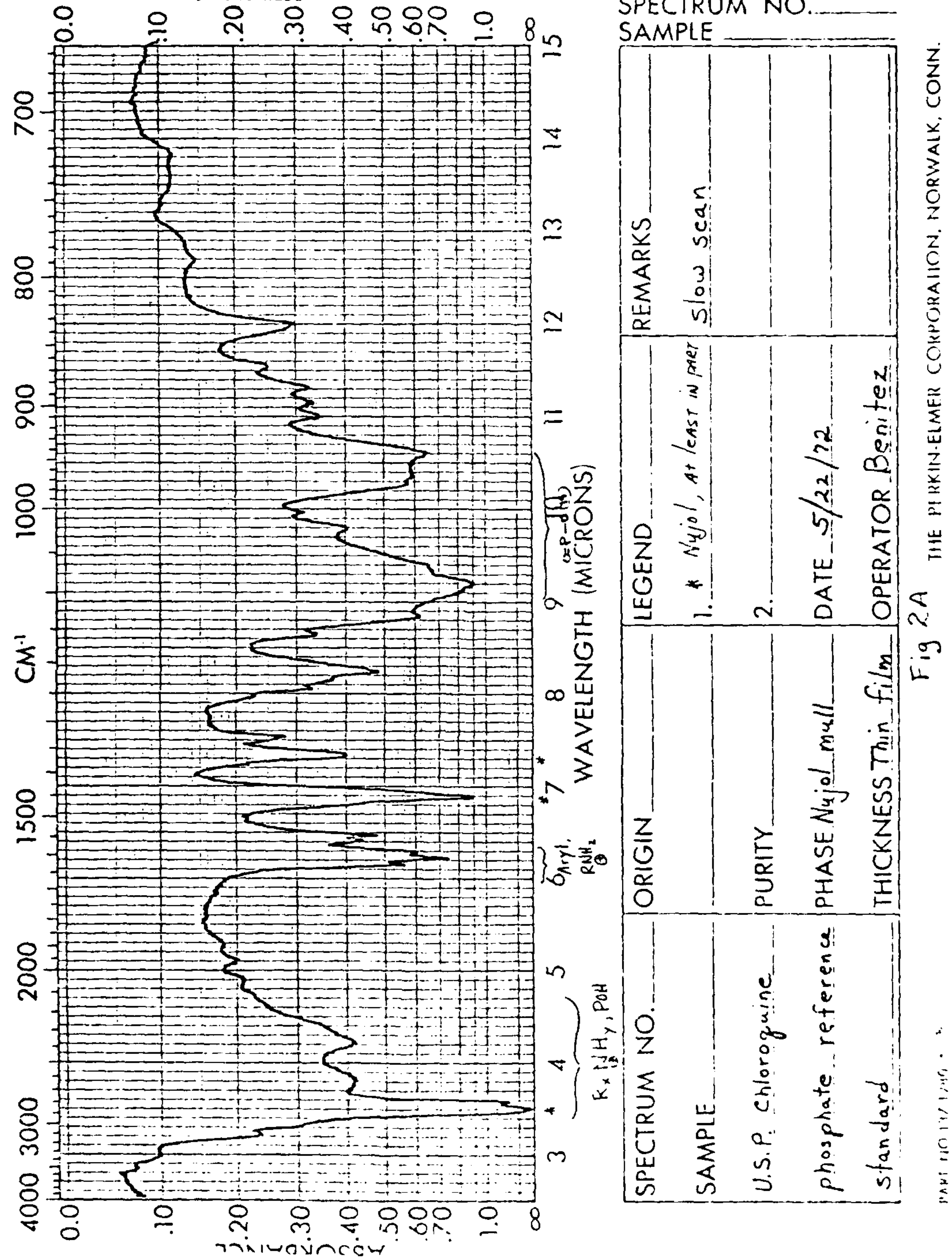
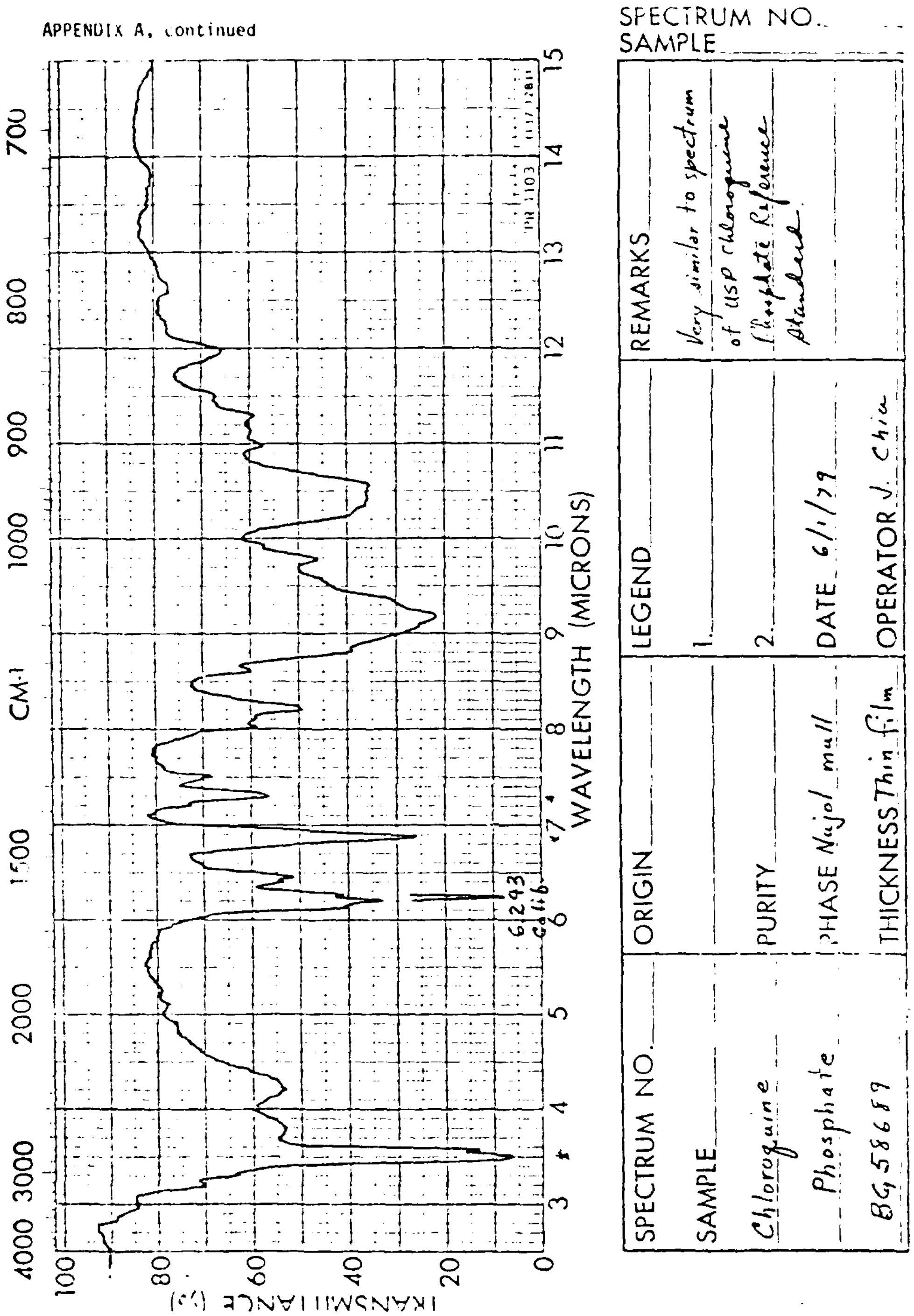
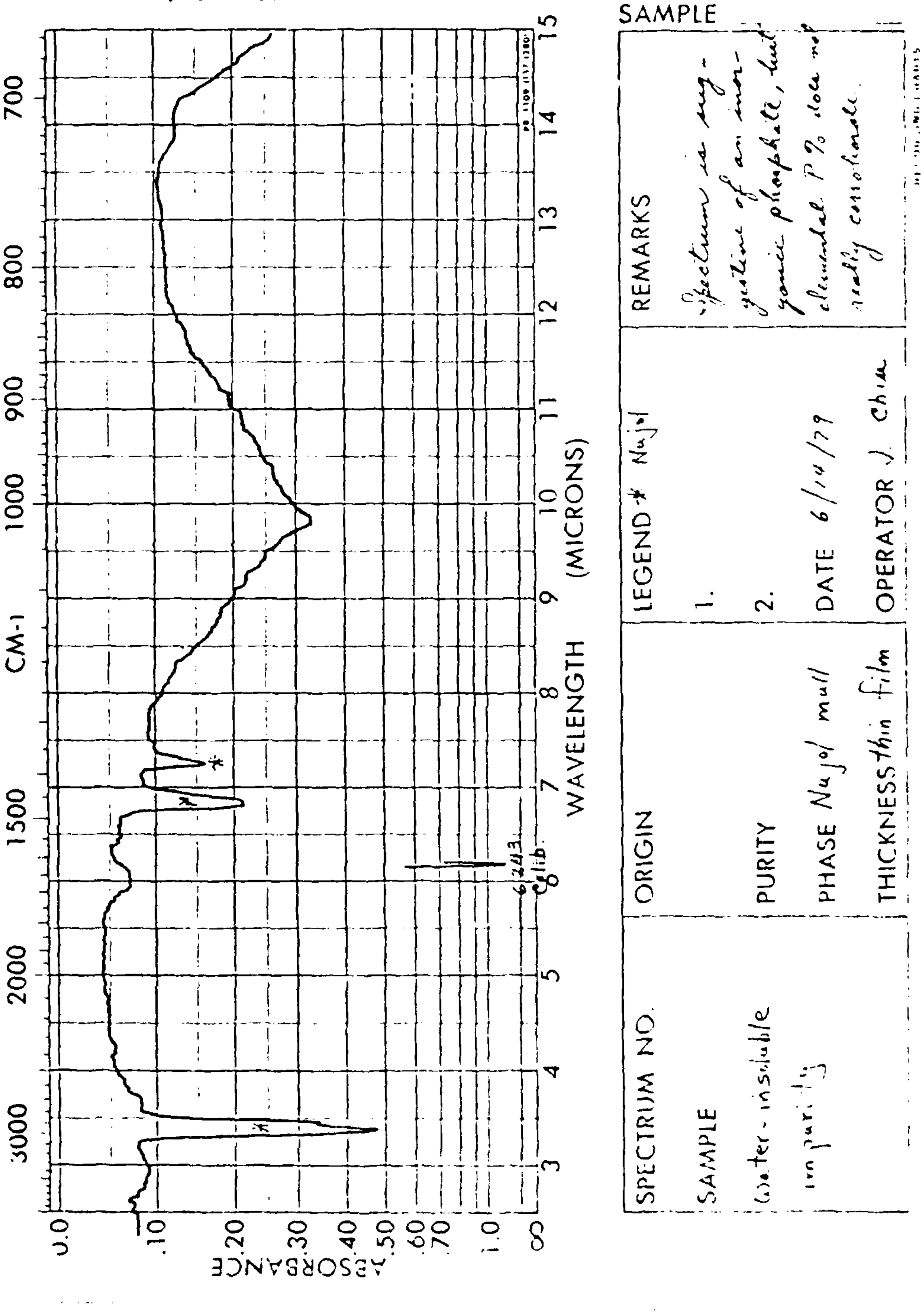


Fig 2A


SPECTRUM NO.
SAMPLE
CM-1
4000 3000 2000 1500 1000 900 800 700
TRANSMITTANCE (%)
100 80 60 40 20 0
WAVELENGTH (MICRONS)
3 4 5 6 7 8 9 10 11 12 13 14 15
6.243 Calib
SPECTRUM NO.
SAMPLE Chloroquine Phosphate BG 58687
ORIGIN
PURITY
PHASE Nujol mull
THICKNESS Thin film
LEGEND
1.
2.
DATE 6/1/79
OPERATOR J. Chia
REMARKS Very similar to spectrum of USP chloroquine Phosphate Reference Standard.

APPENDIX A, continued



LIST OF TABLES

APPENDIX B

TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
100	NG	G	NG	14.11 mm	NG	+
1537	NG	14.11 mm	NG	13.86 mm	NG	+
WT	G	NA	G	NA	NA	+

STERILITY CONTROL

His-Bio Mix   Initial: NG   End: NG   MGA Plate: NG

Top Agar   Initial: NG   End: NG

Diluent: NG   Nutrient Broth: NG

Test Compound (a) Chloroquine NG (b) WR158122 NG (c) NA (d) NA (e) NA

G = Growth   NG = No Growth   NT = Not Tested   NA = Not Applicable   WT = Wild Type

Spontaneous Revertants: TA 100, No S-9, Avg - 79

(1) + = expected response   - = unexpected response

Study Number: 81031/81032   Date: 1 Oct 81   By: Sauers

TABLE 2

TOXICITY LEVEL DETERMINATION

Substance assayed: Chloroquine (WR 1544)   Substance dissolved in: H_2O

Study Number: 81031   Date: 1 Oct 81   Performed by: Sauers, Mullen, Dacey

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 mg/plate	65	72	76	71	NL
10^{-1} mg/plate	56	62	75	64	NL
10^{-2} mg/plate	68	68	73	70	NL
10^{-3} mg/plate	75	69	54	66	NL
10^{-4} mg/plate	49	51	72	57	NL
10^{-5} mg/plate	65	66	54	62	NL
10^{-6} mg/plate	53	71	69	64	NL
10^{-7} mg/plate	61	55	77	64	NL

(1) NG = No Growth   ST = Slight Growth   NL = Normal Lawn

TABLE 3

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	14.13 mm	NG	+
100	NG	G	NG	14.89 mm	NG	+
1535	NG	NA	NG	13.56 mm	NG	+
1537	NG	16.11 mm	NG	13.87 mm	NG	+
1538	NG	NA	NG	13.93 mm	NG	+
WT	G	NA	G	NA	NA	+

STERILITY CONTROL

His-Bio Mix   Initial: NG   End: NG   Diluent: NG

Top Agar   Initial: NG   End: NG   MGA Plate: NG

S-9 Mix   Initial: NG   End: NG   Nutrient Broth: NG

Test Compound (a) Chloroquine NG (b) WR158122 NG (c) NA (d) NA (e) NA (f) NA

G = Growth   NG = No Growth   NT = Not Tested   NA = Not Applicable   WT = Wild Type

(1) + = expected response
- = unexpected response

Study Number: 81031/81032   By: Sauers, Mullen, Dacey, Kellner

Date: 6 Oct 81

TABLE 4

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
AF	2 ug/plate	yes	(329,265,288) 294	(160,283,235) 226			(189,310,383) 294
BP	2 ug/plate	yes	(141,138,225) 168	(177,197,171) 182		(69, 44,106) 73	(44, 38, 78) 53
DMBA	20 ug/plate	yes	(36, 30, 42) 36	(87, 93,107) 96		(12, 8, 12) 11	(24, 19, 10) 18
MNNG	2 ug/plate	no		(446,498,413) 452			
	20 ug/plate	no			(311,309,352) 324		
Spontaneous Reversion Rate							
	before after	no	(20, 14, 9) (NG, NG, NG) 14	(62, 65, 64) (NG, NG, NG) 64	(7, 6, 10) (NG, NG, NG) 8	(4, 2, 7) (NG, NG, NG) 4	(8, 14, 7) (NG, NG, NG) 10
	before after	yes	(14, 14, 9) (NG, NG, NG) 12	(66, 79, 74) (NG, NG, NG) 73	(10, 13, 8) (NG, NG, NG) 10	(2, 4, 5) (NG, NG, NG) 4	(7, 5, 7) (NG, NG, NG) 6

NG = no growth

Study Number: 81031/81032

Date: 6 Oct 81 By: Sauers, Dacey, Kellner, Mullen

TABLE 5

NUMBER OF REVERTANTS/PLATE

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
Chloroquine	1 mg/pl	no	(9, 14, 16) 13	(78, 73, 81) 77	(8, 3, 6) 6	(7, 5, 6) 6	(6, 5, 11) 7
		yes	(6, 14, 9) 10	(62, 57, 52) 57	(6, 4, 6) 5	(4, 6, 3) 4	(10, 13, 18) 14
	0.2 mg/pl	no	(9, 8, 13) 10	(68, 79, 61) 69	(11, 13, 4) 9	(3, 5, 3) 4	(11, 5, 8) 8
		yes	(10, 13, 10) 11	(67, 52, 57) 59	(5, 7, 7) 6	(5, 3, 5) 4	(11, 12, 13) 12
	0.04 mg/pl	no	(9, 8, 10) 9	(51, 64, 69) 61	(8, 9, 8) 8	(3, 5, 4) 4	(10, 8, 7) 8
		yes	(11, 16, 8) 12	(46, 46, 51) 48	(4, 9, 5) 6	(6, 3, 3) 4	(5, 13, 11) 10
	0.008 mg/pl	no	(6, 7, 3) 5	(49, 34, 52) 45	(4, 7, 8) 6	(2, 3, 6) 4	(NG, NG, NG) NG
		yes	(10, 19, 5) 11	(52, 58, 59) 56	(5, 10, 11) 9	(4, 6, 2) 4	(10, 14, 15) 13

-continued

Study No.: 81031 Date: 6 Oct 81 Performed by: Sauers, Kellner, Mullen, Dacey

TABLE 5, concluded

NUMBER OF REVERTANTS/PLATE

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
Chloroquine	0.0016 mg/pl	no	(12, 12, 9) 11	(81, 60, 63) 68	(15, 10, 8) 11	(9, 2, 2) 4	(9, 3, 4) 5
		yes	(11, 12, 4) 9	(54, 51, 31) 45	(4, 2, 2) 3	(3, 7, 2) 4	(14, 10, 4) 9
	0.00032 mg/pl	no	(4, NG, NG) 4	(53, 64, 59) 59	(2, 6, 5) 4	(3, 2, 4) 3	(2, 6, 2) 3
		yes	(16, 11, 13) 13	(76, 54, 51) 60	(5, 4, 11) 7	(2, 3, 5) 3	(9, 7, contam) 8

NG = no growth

Study No.: 81031 Date: 6 Oct 81 Performed by: Sauers, Kellner, Mullen, Dacey

TABLE 6

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	13.94 mm	NG	+
100	NG	G	NG	14.06 mm	NG	+
1535	NG	NA	NG	13.56 mm	NG	+
1537	NG	15.89 mm	NG	12.89 mm	NG	+
1538	NG	NA	NG	13.21 mm	NG	+
WT	G	NA	G	NA	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound (a) Chloroquine NG (b) WR158122 NG (c) NA (d) NA (e) NA (f) NA

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Study Number: 81031/81032 By: Sauers

Date: 21 Oct 81

(1) + = expected response
- = unexpected response

TABLE 7

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
AF	2 ug/plate	yes	(493,506,488) 496	(476,446,311) 411			(677,677,545) 633
BP	2 ug/plate	yes	(83,125,115) 108	(485,523,439) 482		(62, 78, 84) 75	(83, 84,115) 94
DMBA	20 ug/plate	yes	(51, 44, 74) 56	(244,277,245) 255		(15, 17, 24) 19	(27, 22, 22) 24
MNNG	2 ug/plate	no		(999,999,999)* 999			
	20 ug/plate	no			(999,999,999)* 999		
Spontaneous Reversion Rate							
	before after	no	(20, 28, 25) (16, 24, 22) 23	(98, 89, 96) (104,123,101) 102	(15, 13, 14) (12, 15, 17) 14	(7, 9, 6) (6, 7, 9) 7	(17, 12, 11) (11, 12, 15) 13
	before after	yes	(27, 22, 20) (18, 29, 26) 24	(95, 80, 94) (117,121,126) 106	(11, 12, 11) (12, 19, 22) 15	(5, 6, 2) (4, 6, 7) 5	(12, 15, 16) (19, 17, 26) 18

* A value of 999 represents a count of over 1000

Study Number: 81031/81032

Date: 22 Oct 81 By: Sauers, Dacey, Kellner, Mullen

TABLE 8

NUMBER OF REVERTANTS/PLATE

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
Chloroquine	1 mg/pl	no	(18, 26, 30) 25	(100,102,108) 103	(19, 15, 17) 17	(8, 9, 7) 8	(17, 19, 23) 20
		yes	(38, 38, 23) 33	(100, 97, 96) 98	(23, 19, 22) 21	(4, 5, 5) 5	(23, 28, 26) 26
	0.2 mg/pl	no	(19, 13, 15) 16	(104, 81, 95) 93	(20, 18, 18) 19	(14, 15, 15) 15	(22, 23, 18) 21
		yes	(35, 19, 20) 25	(104, 98, 84) 95	(14, 12, 15) 14	(7, 12, 15) 11	(25, 20, 20) 22
	0.04 mg/pl	no	(29, 24, 24) 26	(118,124,116) 119	(20, 19, 22) 20	(14, 15, 12) 14	(25, 27, 23) 25
		yes	(34, 39, 31) 35	(127,127,124) 126	(24, 31, 18) 24	(7, 20, 5) 11	(28, 31, 27) 29
	0.008 mg/pl	no	(30, 27, 25) 27	(103,119, 99) 107	(24, 24, 13) 20	(5, 3, 4) 4	(22, 24, 26) 24
		yes	(34, 39, 27) 33	(131,117,121) 123	(14, 22, 18) 18	(7, 9, 6) 7	(23, 29, 20) 24

-continued

Study No.: 81031 Date: 22 Oct 81 Performed by: Sauers, Dacey, Mullen, Kellner

TABLE 8, concluded

NUMBER OF REVERTANTS/PLATE

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
Chloroquine	0.0016 mg/pl	no	(19, 18, 23) 20	(105,118,121) 115	(16, 14, 13) 14	(6, 7, 3) 5	(18, 20, 12) 17
		yes	(26, 35, 26) 29	(102,119,138) 120	(16, 14, 19) 16	(7, 5, 5) 6	(26, 24, 34) 28
	0.00032 mg/pl	no	(30, 13, 19) 21	(126,102,117) 115	(19, 16, 18) 18	(3, 5, 2) 3	(13, 20, 22) 18
		yes	(36, 29, 30) 32	(127,108,124) 120	(18, 19, 17) 18	(4, 5, 9) 6	(17, 26, 26) 23

Study No.: 81031 Date: 22 Oct 81 Performed by: Sauers, Dacey, Mullen Kellner

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